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INSTRUCTION MANUAL

EVC4000

Multi-channel Voltage/Current Clamp

Serial No. _____

www.wpiinc.com

072816

CONTENTS

ABOUT THIS MANUAL 1

INTRODUCTION 2

 Unpacking..... 2

INSTRUMENT DESCRIPTION 2

 System Architecture 2

 Preamplifier 2

 Preamplifier Panel Controls and Connectors 3

 Clamp Modules..... 4

 Clamp Module Front Panel 4

 Clamp Module Rear Panel..... 5

OPERATING INSTRUCTIONS..... 5

 Dry Run..... 5

 Voltage Clamp..... 5

 Fluid Resistance Compensation 6

 Operation..... 7

 Connecting the Chamber 7

 Compensate for Voltage Electrode Asymmetry..... 7

 Compensate Fluid Resistance 7

 Voltage Clamping..... 7

 Alarm 8

 Current Clamp..... 8

 Interface Pinout for EVC4000..... 8

SPECIFICATIONS..... 8

DECLARATION OF CONFORMITY 10

WARRANTY 11

 Claims and Returns 11

 Repairs..... 11

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ABOUT THIS MANUAL

The following symbols are used in this guide:



This symbol indicates a CAUTION. Cautions warn against actions that can cause damage to equipment. Please read these carefully.



This symbol indicates a WARNING. Warnings alert you to actions that can cause personal injury or pose a physical threat. Please read these carefully.

NOTES and TIPS contain helpful information.

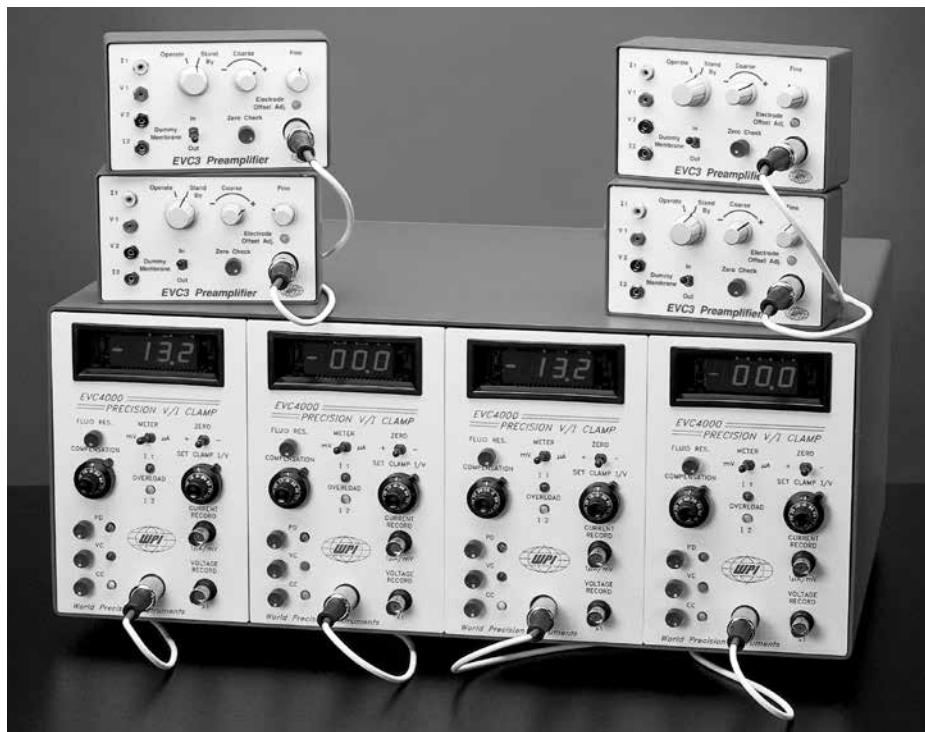


Fig. 1—The EVC4000

INTRODUCTION

A multi-channel Voltage/Current clamp apparatus for the study of the electrical properties of epithelial tissues, **EVC4000** employs the voltage clamp technique to study temporal changes in membrane permeability as a function of membrane voltage or applied chemical agents. This instrument can measure up to four tissue samples simultaneously.

Each module, with its companion preamplifier, can operate independently in one of three different modes: Voltage Clamp (VC), Current Clamp (CC) or Open Circuit Potential (PD) measurement. Operation of the EVC4000 can be effected manually from the front panel of the instrument or from computer generated commands applied at the rear panel of the instrument.

Unpacking

Upon receipt of this instrument, make a thorough inspection of the contents and check for possible damage. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed damage should be reported at once to the carrier and an inspection requested. Please read the section entitled "Claims and Returns" on page 11 of this manual. Please contact WPI Customer Service if any parts are missing at 941.371.1003 or customerservice@wpiinc.com.

Returns: Do not return any goods to WPI without obtaining prior approval (RMA # required) and instructions from WPI's Returns Department. Goods returned (unauthorized) by collect freight may be refused. If a return shipment is necessary, use the original container, if possible. If the original container is not available, use a suitable substitute that is rigid and of adequate size. Wrap the instrument in paper or plastic surrounded with at least 100mm (four inches) of shock absorbing material. For further details, please read the section entitled "Claims and Returns" on page 11 of this manual.

INSTRUMENT DESCRIPTION

System Architecture

Preamplifier

Each channel module of **EVC4000** provides power to its own remote preamplifier (see Figure 1) connected by a flexible cable to the front panel. Preamplifiers should be mounted close to the site of the experiment. To avoid accidental wetting of its electronic circuits, the preamplifier should be supported on a ring stand mount so that its front face is in a vertical position. Membranes to be tested are normally placed into Ussing chambers or similar devices. One chamber of a test membrane can be arbitrarily designated as the "1" side and the transmural chamber as the "2" side. Four electrodes are positioned so that current electrode I_1 and voltage electrode V_1 are placed in chamber 1; electrodes I_2 and V_2 in chamber 2.

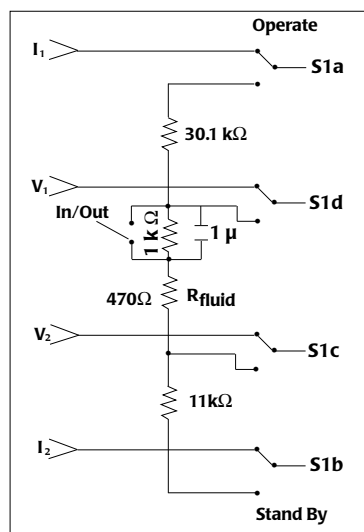
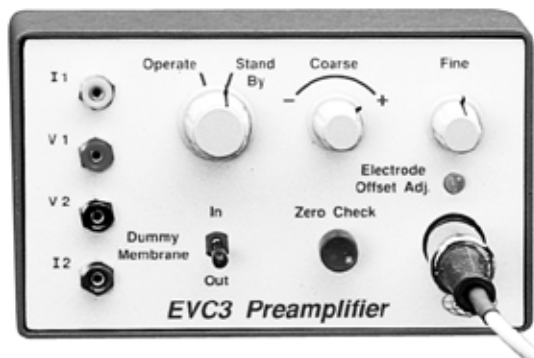


Fig. 2—(Left) The preamplifier.

Fig. 3—(Right) Resistance in Stand By Mode – 41.57 KΩ.

A unique feature of the EVC4000 preamplifier is a local circuit that maintains the potential of the V2 electrode invariant at zero potential relative to the system ground. Thus the preamplifier apparatus will actively maintain the “2” surface of the test membrane close to ground potential under all operating conditions. This is accomplished by incorporating an electronic potentiostat in the preamplifier box.

Preamplifier Panel Controls and Connectors

Operate/Stand By: In **Operate** mode, input electrode connectors I_1 , V_1 , I_2 , and V_2 are connected to the preamplifier. In **Stand By** mode, the preamplifier is disconnected from external electrodes and is connected instead to a resistor network (See Fig. 3).

Electrode Offset Adj: Two variable controls, Coarse and Fine, with which to adjust any small voltage asymmetry between voltage electrodes V_1 and V_2 to zero. Once adjusted, the user should not move these controls during an experiment.

Zero Check: Amplifier input is grounded.

Dummy Membrane: In the Stand By operating mode when the toggle switch is toggled to “In”, a resistor and shunt capacitor, simulating a membrane, is inserted between the inputs to the membrane voltage amplifier. “Out” removes this circuit.

Clamp Modules

Up to four clamp modules (Fig. 4) can be housed in the main control housing which supplies power for all modules and preamplifiers.

Fig. 4—(Right)

Clamp Module Front Panel

Digital Panel Meter: The digital meter on each control section is a 3½-digit light emitting diode (LED) display. Using the Meter Select switch, membrane voltage in millivolts or trans-membrane current flow in microamperes will be displayed.

FLUID RES COMPENSATION: This ten-turn dial allows the user to dial a resistance value, empirically determined, that enables the voltage clamp to compensate the voltage error that is caused by clamp current flowing through the electrolyte path between the voltage electrodes and the membrane surface. The push button injects an arbitrary DC current through the chamber (in the absence of the membrane) to allow the user to determine fluid resistance.

SET CLAMP I/V: This ten turn dial allows the user to set: a) the clamp voltage or b) a constant current. Each full rotation of the dial is equivalent to 10 millivolts or 100 μ amperes of current. The **ZERO, +/-** toggle switch above the potentiometer selects the polarity of the clamping voltage (or current). Mid position of the toggle switch results in zero volts or microamperes. Polarity Convention: Membrane Potential is defined as plus if V_1 of the voltage clamp apparatus is positive relative to V_2 . Current is defined as plus if + ion current flows from electrode I_1 to I_2 .

PD, VC, CC: Push buttons, adjacent to indicator lamps, initiate one of three different operating modes; PD (open circuit membrane potential difference), CC (constant current or "current clamp") or VC (voltage clamp).

VOLTAGE RECORD: An output connector for display of membrane voltage on a recorder, computer or oscilloscope.

CURRENT RECORD: An output connector for display of current magnitude and polarity (1 millivolt = 1 microampere).

OVERLOAD: Lamps I_1 and I_2 , when lit, indicate that a limit has been reached in the circuit serving the current electrodes. This may be due to excessive clamp current, a defective electrode or an open circuit in the current electrode pathway. An audible alarm sounds if either lamp is lit.



Clamp Module Rear Panel

POWER Switch: When the Power Switch is turned on, the instrument automatically assumes the open circuit potential difference, **PD**, state as indicated by the green lamp next to the PD button.

REMOTE LOGIC: Positive 5 volts logic level applied to either **VC** or **CC**, **REMOTE LOGIC** input connectors, will assert the VC or CC operating mode respectively for **as long as the logic level** is sustained. When the logic level applied to VC or CC ceases, **PD operating mode will be automatically asserted.**

EXT CLAMP COMMAND: Externally applied voltages can be applied to either set transmembrane voltage in the VC (voltage clamp) mode or to set transmembrane current in the CC (current clamp) mode. The amplitude and polarity of the command determines the amplitude and polarity of the resulting membrane voltage or current. **Panel selected SET CLAMP values (of current or voltage) are superimposed upon the externally applied command unless the front panel SET CLAMP I/V toggle switch is in the OFF position. See specifications for command factors converting applied voltage to clamp voltage or current.**

OPERATING INSTRUCTIONS

Dry Run

An excellent way to become familiar with the operation of the **EVC4000** is to simulate voltage and current clamping. The electrical performance of the instrument can also be determined.

Connect the preamplifier cable to the receptacle of any available module on the front panel of the instrument. Place the Operate/Stand By switch in Stand By position and the Dummy In/Out toggle switch In. This causes dummy electrodes composed of resistors, as shown in Fig. 3 to be inserted across the probe input connector array. Connect the instrument power cord to the power line and turn the instrument power on using the rear panel power switch. Two green lamps should light; one adjacent the PD push button and another on the preamplifier panel. Set the METER switch to mV. Push the ZERO CHECK button on the probe and note that the digital panel meter will display zero mV. Releasing the button allows the Coarse and Fine adjustment knobs to control the input offset voltage. Note that rotating these knobs will cover a range of approximately $\pm 30\text{mV}$ allowing the user to adjust small voltage electrode asymmetries to zero. Adjust the offset voltage so that the meter reads 0.0mV; do not adjust the offset voltage further.

Voltage Clamp

Push the VC button. The red lamp next to the button should light, indicating that the instrument is in the voltage clamp operating mode. Keep the FLUID RES. COMPENSATION knob at zero. Place the polarity toggle switch in the + position and rotate the SET CLAMP I/V knob. Note that one full turn indicates 10.0mV on the

digital meter. Reversing the polarity toggle switch will yield -10.0 mV. Rotate the SET CLAMP I/V knob until a voltage of about 14.7mV is reached. Switch METER SELECT to microamperes (mA), a clamping current of 10 (+/-1) microamperes will result. To test the alarm for maximum clamp current of 1mA, place the Dummy In/Out toggle switch at Out. This essentially connects **EVC4000** to a 470 Ω resistor, which is used to simulate fluid resistance, as shown in Figure 2 on page 2. Rotate the FLUID RES. COMPENSATION dial knob for approximate four full turns until a reading of 4.0 is displayed on the dial. Now, turn the METER SELECT back to mV position and then rotate the SET CLAMP I/V knob to increase the set voltage. At about 70mV of set voltage, at which a clamping current of 1 mA is required, alarm lamps and audible signals are activated.

Fluid Resistance Compensation

The purpose of the procedure below, in a real experiment, is to prevent varying transmural current (during voltage clamp) from causing an error in the measured transmural voltage. It allows the user to experimentally determine the resistance of bathing fluid normally between the voltage electrodes and the surfaces of a membrane. The measurement, performed in the absence of a membrane, allows the experimenter to exactly compensate errors in voltage clamping caused by fluid resistance.

1. Using FLUID RES button:

Push the PD button. The green lamp next to the button should light. Place the METER switch on mV. Switch Dummy Membrane to Out. Push the FLUID RES. switch. This causes the voltage reading to increment. Advance the FLUID RES COMPENSATION control so that the meter voltage reads 0.0 mV. The dial reading of the FLUID RES COMPENSATION knob assembly will then read the "fluid resistance" which exists between electrodes V1 and V2. In this case, 470 $\Omega \pm 10\%$. Once fluid resistance has been measured, the control is left at that value and the user may then be assured that little error will result as a consequence of current flow during a voltage clamp experiment.

2. Using Constant Current mode:

The constant current, CC, mode enables the user to cause a known current to flow through the membrane sample (in this case the dummy resistor circuit). Polarity and magnitude are determined by the polarity toggle switch and the SET CLAMP I/V control, respectively.

On the preamplifier, switch Dummy Membrane to Out. Push the CC button. The yellow lamp adjacent to the CC button should light. Place METER switch on μ A. Set SET CLAMP toggle switch polarity switch to either + or - and dial SET CLAMP I/V control to approximately 50 μ A as indicated on the digital panel meter. Switch METER to mV. Advance the FLUID RES COMPENSATION control so that the meter voltage reads 0.0 mV. The dial reading of the FLUID RES COMPENSATION knob assembly will then read

the “fluid resistance” which exists between electrodes V1 and V2 . In this case, $470\Omega \pm 10\%$. Once fluid resistance has been compensated, the user may then be assured that little error will result as a consequence of current flow. Notice that rotating the SET CLAMP I/V knob (i.e., varying the DC current) will not significantly vary “membrane volts” when the FLUID RES COMPENSATION has been optimally adjusted to exactly equal the “fluid “ resistance.

Operation

Maintain the EVC4000 preamplifiers in Stand By when the instrument is not in use or while electrodes are being connected.

Connecting the Chamber

Assemble the membrane test chamber without a membrane. Two voltage electrodes*, one on either side of where the membrane is normally located are inserted into the appropriate ports on the (Ussing) chamber. Two current electrodes* are also connected at ports located at each distal end of their respective chambers. Note that “1” electrodes, i.e., V1 and I1 are connected on the same chamber half; similarly for “2” chamber electrodes, V2 and I2. Fill the chamber with electrolyte through the chamber circulation ports.

Compensate for Voltage Electrode Asymmetry

In the PD operating mode, switch the Stand By/Operate switch to Operate. Adjust the Electrode Offset Adj. controls on the preamplifier so as to reduce the asymmetry potential to 0.0mV.

Compensate Fluid Resistance

Depress the FLUID RES. test button and at the same time advance the FLUID RES. COMPENSATION knob so as to reduce the panel meter reading to 0 mV. Return Stand By/Operate switch to Stand By. Disassemble the chamber, install the tissue sample, reassemble and refill with electrolyte. Proceed with V/I clamp experiments.

Voltage Clamping

Maintain the Preamplifier in Stand By mode while making front panel adjustments. It is always good procedure to first check the open circuit transmural potential difference (PD). If the reading polarity and DC amplitude is normal for the membrane sample, one can continue confidently to Voltage Clamp. If the green PD lamp is not lit, push the PD button. Switch from Stand By to Operate on the preamplifier. Return to Stand By after observing the potential (mV) on the panel meter. Use the SET CLAMP I/V controls to set the polarity and amplitude of the clamp voltage. For example, if a “short circuit” clamp is desired place the toggle switch to its center ZERO position. Push the VC button, red lamp will light. Voltage reading should be 0 for short circuit clamp. Switch meter from mV to μA and observe the membrane clamp current. Voltages proportional to clamp voltage and clamp current are continuously available at VOLTAGE RECORD AND CURRENT RECORD output connectors respectively.

Alarm

The maximum internal voltage available with which current can be forced to flow through the test chamber is $\pm 32\text{V}$. If, during voltage clamping, the external current path is accidentally disconnected or if the resistance of this path is too high for the appropriate clamp current to flow, I1 lamp will light and an audible alarm will sound. Alternatively, if the current exceeds the maximum current available (i.e., $1000\mu\text{A}$) I2 lamp will light, and the alarm will sound.

Current Clamp

In the Stand By mode, set the polarity toggle switch and value of current desired on the ten turn knob. Set the meter to display either current or membrane voltage as desired. Switch to Operate mode and observe and record results.

Interface Pinout for EVC4000

	CH1	CH2	CH3	CH4
Command	30	18	14	2
V Monitor	31	19	15	3
I Monitor	32	20	16	4
V Clamp	26	23	18	7
I Clamp	27	24	11	8
GND	1,5,6,9,12,13,17,21,22,25,28,29,33			

SPECIFICATIONS

This unit conforms to the following specifications:

Preamplifier:

Input Resistance	$10^{12} \Omega$
Input Leakage Current	100 pA, max.
Absolute Maximum Input Voltage.....	$\pm 15\text{V}$

Voltage Clamp:

Panel Setting Range	$\pm 200 \text{ mV}$
Clamp V. per Volts Applied External	100mV per V (ie., $1.93\text{V}=193\text{mV}$ clamped)
Max. Voltage Range of Current Electrodes	$\pm 32\text{V}$
Max. Clamp Current*	1 mA
Fluid Resistance Compensation	0 to 1000Ω

Current Clamp:

Maximum Current.....	+ 1mA
Current per External Volts Applied	$1\mu\text{A}$ per mV (ie., An input of $50\text{mV}=50\mu\text{A}$ CC)

DECLARATION OF CONFORMITY



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DECLARATION OF CONFORMITY

We: World Precision Instruments, Inc.
175 Sarasota Center Boulevard
Sarasota FL 34240-9258
USA

as the manufacturers of the apparatus listed, declare under sole responsibility that the product(s):

Title: EVC4000-4
(also covering models EVC4000-1, EVC4000-2 and EVC4000-3; complete with Probe Preamplifier Type EVC3)

to which this declaration relates is/are in conformity with the following standards or other normative documents:

Safety: EN 61010-1:1993 (IEC 1010-1:1990)

EMC: EN 50081-1:1992
EN 50082-1:1992

and therefore conform(s) with the protection requirements of Council Directive 89/336/EEC relating to electromagnetic compatibility and Council Directive 73/23/EEC relating to safety requirements.

Issued on: 18th February 2000



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WARRANTY

WPI (World Precision Instruments, Inc.) warrants to the original purchaser that this equipment, including its components and parts, shall be free from defects in material and workmanship for a period of one year* from the date of receipt. WPI's obligation under this warranty shall be limited to repair or replacement, at WPI's option, of the equipment or defective components or parts upon receipt thereof f.o.b. WPI, Sarasota, Florida U.S.A. Return of a repaired instrument shall be f.o.b. Sarasota.

The above warranty is contingent upon normal usage and does not cover products which have been modified without WPI's approval or which have been subjected to unusual physical or electrical stress or on which the original identification marks have been removed or altered. The above warranty will not apply if adjustment, repair or parts replacement is required because of accident, neglect, misuse, failure of electric power, air conditioning, humidity control, or causes other than normal and ordinary usage.

To the extent that any of its equipment is furnished by a manufacturer other than WPI, the foregoing warranty shall be applicable only to the extent of the warranty furnished by such other manufacturer. This warranty will not apply to appearance terms, such as knobs, handles, dials or the like.

WPI makes no warranty of any kind, express or implied or statutory, including without limitation any warranties of merchantability and/or fitness for a particular purpose. WPI shall not be liable for any damages, whether direct, indirect, special or consequential arising from a failure of this product to operate in the manner desired by the user. WPI shall not be liable for any damage to data or property that may be caused directly or indirectly by use of this product.

Claims and Returns

Inspect all shipments upon receipt. Missing cartons or obvious damage to cartons should be noted on the delivery receipt before signing. Concealed loss or damage should be reported at once to the carrier and an inspection requested. All claims for shortage or damage must be made within ten (10) days after receipt of shipment. Claims for lost shipments must be made within thirty (30) days of receipt of invoice or other notification of shipment. Please save damaged or pilfered cartons until claim is settled. In some instances, photographic documentation may be required. Some items are time-sensitive; WPI assumes no extended warranty or any liability for use beyond the date specified on the container

Do not return any goods to us without obtaining prior approval and instructions from our Returns Department. Goods returned (unauthorized) by collect freight may be refused. Goods accepted for restocking will be exchanged or credited to your WPI account. Goods returned which were ordered by customers in error are subject to a 25% restocking charge. Equipment which was built as a special order cannot be returned.

Repairs

Contact our Customer Service Department for assistance in the repair of apparatus. Do not return goods until instructions have been received. Returned items must be securely packed to prevent further damage in transit. The Customer is responsible for paying shipping expenses, including adequate insurance on all items returned for repairs. Identification of the item(s) by model number, name, as well as complete description of the difficulties experienced should be written on the repair purchase order and on a tag attached to the item.

** Electrodes, batteries and other consumable parts are warranted for 30 days only from the date on which the customer receives these items.*

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